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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Benson *et al.*

Application No.: 08/942,067 Group Art Unit: 3615

Filed: October 1, 1997 Examiner: Houtteman

For: AROMATIC-SUBSTITUTED XANTHENE DYES Attorney Docket No.: 4356

Amendment Under 37 CFR §1.116

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants have carefully considered the final Office Action dated February 17, 1999 in connection with the captioned application. Reconsideration of the claims in light of the amendments and remarks that follow is kindly solicited.

Amendment

In the Claims

In Claim 1, in the ultimate line of the definition of substituent R_1, delete "and indene".

In Claim 8, delete each occurrence of "napthyl" and insert therefor --naphthyl--.

In Claim 9, delete each occurrence of "napthyl" and insert therefor --naphthyl--.

In Claim 12, delete "floro" and insert therefor --fluoro--.

In Claim 17, delete "napthyl" and insert therefor --naphthyl--.

In Claim 35, lines 2, 10 and 12, delete "forth" and insert therefor --fourth--.

Remarks

With this response, Claims 1, 8, 9, 12, 17 and 35 have been amended without prejudice against Applicants' right to pursue claims drawn to the amendatory subject matter in one or more timely filed continuation, divisional or continuation-in-part applications. No claims have

been deleted or added. Claims 1-37 stand rejected under 35 USC § 103(a) as being unpatentable over Lee *et al.*, 1992, *Nucl. Acids Res.* **20**(10):2471-2483 ("Lee *et al.*").

1. The Amendments of the Claims

Claims 1, 8, 9, 12, 17 and 35 have been amended to more clearly define Applicants' invention and/or to correct obvious typographical errors. Specifically, Claim 1 has been amended to delete the alternative "indene" from the definition of substituent R_1 when R_1 is taken together with R_7. The amendment is supported by Claim 1 as originally filed and in the specification at page 12, lines 13-21. The amendments of Claims 8, 9 and 17 merely correct obvious errors in the spelling of "naphthyl." Similarly, the amendments of Claims 12 and 35 correct obvious errors in the spelling of "fluoro" and "fourth," respectively. As the amendments do not introduce new matter and place the claims in condition for allowance, entry thereof is respectfully requested.

2. Rejection of Amendment Submitted 12/2/98 Under 35 USC § 132

The amendments of structures V.1 and V.2 (at page 20 of the specification) submitted December 2, 1998 were not entered as allegedly constituting "new matter." Applicants submit these amendments merely correct obvious typographical errors and are therefore fully supported by the specification as originally filed.

An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of an error in the specification, but also the appropriate correction. *In re Oda*, 170 USPQ 260 (CCPA 1971). The amendments of structures V.1 and V.2 fall into this category.

It is clear from the disclosure at pages 19 and 20 that structures V.1 and V.2 illustratively describe certain preferred embodiments of structure V. Structure V falls under the heading "Phosphoramidite Reagents" and correctly illustrates a phosphoramidite. As evidenced by the attached copy of the Beaucage & Caruthers paper which first introduced phosphoramidite synthesis reagents (Beaucage & Caruthers, 1981, *Tetrahdron Lett.* **22**(20):1859-1862; Exhibit A), phosphoramidites were a well-recognized class of compounds in the art of organic and/or DNA synthesis chemistry at the time the instant application was filed. As a consequence, not only would those of skill in the art recognize the existence of errors in structures V.1 and V.2 at the P and N atoms, they would also recognize the appropriate corrections as being the amendments submitted by Applicants on December 2, 1998. Accordingly, as the amendments submitted December 2, 1998 merely correct obvious errors in the specification, entry thereof is proper and respectfully requested.

3. Rejection Under 35 USC § 103(a)

Claims 1-37 stand rejected under 35 USC § 103(a) as being obvious over Lee *et al.* for the reasons of record. The crux of the rejection appears to lie in the notion that Lee *et al.* teach dye molecules that are structurally similar homologs of the instantly claimed dye molecules. Applicants traverse the rejection on the grounds that the Examiner has failed to establish a *prima facie* case of obviousness.

3.1 The Legal Standard of *Prima Facie* Obviousness

When rejecting claims under 35 USC § 103, the Examiner bears the burden of establishing a *prima facie* case of obviousness. *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993). To establish a *prima facie* case, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify or combine the reference teachings in the manner suggested by the Examiner. *See, e.g., In re Grabiak*, 226 USPQ 870 (Fed. Cir. 1985). Second, the skilled artisan, in light of the teachings of the prior art, must have a reasonable expectation that the modification or combination suggested by the Examiner would be successful. *See e.g., In Re Dow*, 5 USPQ2d 1529, 1531-32 (Fed. Cir. 1988). Finally, the prior art reference, or references when combined, must teach or suggest each and every limitation of the claimed invention. MPEP § 706.02(j). The teaching or suggestion to make the claimed invention *and* the reasonable expectation of success must *both* be found in the prior art, not in the Applicant's disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). If any one of these criteria is not met, *prima facie* obviousness is not established and the rejection must be withdrawn.

The Examiner appears to be of the notion that the burden of establishing *prima facie* obviousness is discharged by merely alleging, without more, "structural obviousness" between the prior art and claimed compounds. A careful review of the relevant law reveals this is not the case. As stated by the Federal Circuit in *In re Grabiak*:

> [G]eneralization should be avoided insofar as specific chemical structures are alleged to be *prima facie* obvious one from the other . . . . [T]here must be *adequate support* in the prior art for the . . . change in structure in order to complete the PTO's *prima facie* case and shift the burden of going forward to the applicant.

In re Grabiak, 226 USPQ at 871-872 (emphasis supplied).

In *Grabiak*, Grabiak's claimed thiazole thiocarboxylate herbicidal safener compounds were rejected as being obvious over a combination of two references: one which taught a

structurally similar thiazole carboxylate compound that also had utility as an herbicidal safener and another which taught the interchangeability of oxygen and sulfur in compounds having safening properties. Quite notably, the *only difference* between the prior art compound and the compounds claimed by Grabiak was the presence of a sulfur atom instead of an oxygen atom in the ester moiety of Grabiak's compounds.

Yet, given the very close structural similarities between the prior art and claimed compounds, the Federal Circuit found that *prima facie* obviousness had not been established:

> The PTO cited no pertinent reference showing or suggesting to one of ordinary skill in the art the change of a thioester for an ester group. *In the absence of such a reference, there is inadequate support* for the PTO's position that this modification would *prima facie* have been obvious.

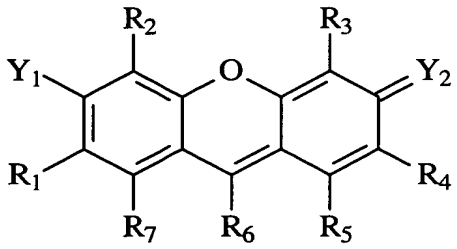
In re Grabiak, 226 USPQ at 872 (emphasis supplied).

Thus, *Grabiak* makes clear that mere allegations of "structural similarity" between prior art and claimed compounds, without out more, are *insufficient* to sustain a conclusion of *prima facie* obviousness. The prior art must *adequately support* the necessary change in structure by *showing or suggesting* to one of ordinary skill in the art *the necessary change*. *Accord Kimberly-Clark v. Johnson & Johnson*, 223 USPQ 223, 610 (Fed. Cir. 1984) (prior art must *clearly suggest* the claimed subject matter).

3.2 The Lee *et al.* Reference Does Not Teach or Suggest the Instantly Claimed Xanthene Dye Compounds

The "support" provided by the cited Lee *et al.* reference falls far short of that required to sustain a *prima facie* case of obviousness. In fact, it does not even rise to the level found to be *inadequate* in *Grabiak*.

As amended, Claim 1 recites a class of aromatic-substituted xanthene dye molecules described by the following generic structural formula:



Quite notably, in all of the claimed compounds, the substituent R_1, when taken alone, is an *aromatic* moiety selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic and electron-rich heterocycle. Alternatively, when taken together with R_7, the fused substitutent is an electron-rich heterocycle. Thus, contrary to the Examiner's assertion, while substituent R_7 may be selected from the group consisting of, among others, chlorine and lower alkoxy, these groups are *not* included amongst the list of alternative substituents that can be used to substitute the xanthene ring at position R_1.

Lee *et al.* teach the behavior of various xanthene- and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene- ("BODIPY") dye-labeled 2',3'-dideoxynucleosides when used in termination sequencing reactions employing T7 DNA polymerase. As evidenced by the following table, which defines the various dye molecules taught by Lee *et al.* using the generic structural formula of amended Claim 1, the structures of the Lee *et al.* dye molecules differ significantly from the aromatic-substituted xanthenes recited in amended Claim 1:[1/]

Compound (FIG. No)	Substituent Variable								
	Y_1	Y_2	R_1	R_2	R_3	R_4	R_5	R_6	R_7
3a	O	O	H	H	H	H	H	Ph[2/]	H
3b	O	O	H	H	H	H	H	Ph	H
3c	O	O	H	H	H	H	H	Ph	H
3d	O	O	H	H	H	H	H	Ph	H
3e	O	O	Cl	H	H	Cl	H	[3/]	H
3f	NH_2	NH_2^+	H	H	H	H	H	Ph	H
3g	NH_2	NH_2^+	H	H	H	H	H	Ph	H
3h/i	NMe_2	NMe_2^+	H	H	H	H	H	Ph	H
4a	O	O	H	H	H	H	H	Ph	H
4b	O	O	Cl	H	H	Cl	H	Ph	H
4c	O	O	Cl	Cl	Cl	Cl	H	Ph	H

[1/] Lee *et al.* Compounds 4g, 5d and 6e are not shown, as these compounds have a BODIPY rather than a xanthene parent ring.

[2/] In all of the compounds, Ph refers to a substituted phenyl. The exact identities and positions of the substituents are not germane to the discussion.

[3/] R_6 is $-CH_2-CH(COO^-)-CH_2-CONH-$

Compound (FIG. No)	Substituent Variable								
	Y_1	Y_2	R_1	R_2	R_3	R_4	R_5	R_6	R_7
4d	O	O	OMe	H	H	OMe	H	Ph	H
4e	O	O	OMe	H	H	OMe	H	Ph	H
4f	O	O	OMe	Cl	Cl	OMe	H	Ph	H
4h	NMe_2	NMe_2^+	H	H	H	H	H	Ph	H
5a	O	O	OMe	Cl	Cl	OMe	H	Ph	H
5b	O	O	OMe	Cl	Cl	OMe	H	Ph	H
5c	O	O	benzeno	Cl	Cl	benzeno	–	Ph	–
5e	EtNH	$EtNH^+$	Me	H	H	Me	H	Ph	H
6a	O	O	H	H	H	H	H	Ph	H
6b	O	O	OMe	H	H	OMe	H	Ph	H
6c	O	O	OMe	Cl	Cl	OMe	H	Ph	H
6d	O	O	benzeno	H	H	benzeno	–	Ph	–
6f	NH_2	NH_2^+	H	H	H	H	H	Ph	H
6g	NMe_2	NMe_2^+	H	H	H	H	H	Ph	H

None of these compounds are homologs of the xanthene dyes recited in amended Claim 1, as alleged by the Examiner.[4/] All of the compounds of Lee *et al.* have either a hydrogen, chlorine, methyl or methoxy substituent at the R_1 position of the ring (when R_1 is taken alone). In stark contrast, the compounds recited in amended Claim 1 have either a phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic or electron-rich heterocycle substitutent at the R_1 position. The only compounds taught by Lee *et al.* in which R_1 and R_7 are taken together are Compounds 5c and 6d. Both of these compounds are substituted with a benzeno group. Compounds recited in amended Claim 1 in which R_1 and R_7 are taken together are substituted with an electron-rich heterocycle. Thus, the Examiner's position that Lee *et al.* teach compounds which are homologs of the instantly claimed compounds is simply untenable.

[4/] Homologs are a series of related compounds that have the same functional group(s) but differ in formula by a fixed group of atoms. For example, alkanes (*e.g.*, methane, ethane, propane, butane, etc.), which differ from one another by the number -of CH_2- groups present, form a homologous series. *See*, A DICTIONARY OF CHEMISTRY, 1996, Oxford University Press, pp. 248 (Exhibit B).

Moreover, nothing in the Lee *et al.* reference teaches or suggests altering the dye molecules disclosed therein at *any* ring positions, let alone the R_1 and/or R_1/R_7 positions, in the manner necessary to arrive at the instantly claimed compounds. Lee *et al.* are completely silent regarding ring substitutions. The authors merely catalogued the behavior of several available dyes to determine whether they would be suitable for use in termination sequencing reactions employing T7 DNA polymerase as the polymerizing enzyme. Clearly, *complete silence* regarding the modifications necessary to arrive at the instantly claimed dye compounds falls well below the level of support required by *Grabiak* to sustain a *prima facie* case of obviousness.

3.3 The Lee et al. References Teaches Away From the Instantly Claimed Xanthene Dye Compounds

If anything, the Lee *et al.* reference *teaches away* from the instantly claimed dye compounds, which is the hallmark of unobviousness. *See, e.g. Kloster Speedsteel AB v. Crucible, Inc.*, 230 USPQ 81 (Fed. Cir. 1986), *on rehearing*, 231 USPQ 160 (Fed. Cir. 1986). At the paragraph bridging pages 2476-2477, the authors teach that within the fluoresceine and rhodamine dye classes tested, "the smaller the dye, the better." While the use of small dyes did not guarantee uniform patterns, the bigger dyes, such as Compounds 4f, 5a and 5b, gave very small peaks in termination reactions in which terminal Ts and Cs followed Gs (Lee *et al.* at page 2477, Col. 1).

It is apparent from studying the various structures of Lee *et al.* in conjunction with the discussion at page 2477, Col. 1, that "small" refers to the substitution pattern of the xanthene ring: the "small" dye 5-FAM (Compound 4a) contains hydrogens at ring positions R_1, R_2, R_3, R_4, R_5 and R_7, whereas the "bigger" dyes 6-JOE (Compound 4f) and LOU (Compounds 5a and 5b) each contain methoxy substituents at positions R_1 and R_4, chloro substituents at positions R_2 and R_3 and hydrogen substituents at positions R_5 and R_7. The aromatic and heterocycle substituents recited in amended Claim 1 are even bigger than the methoxy and chloro substituents of 6-JOE and LOU. As a consequence, in order to modify the dyes of Lee *et al.* in the manner necessary to arrive at the instantly claimed dyes, one of ordinary skill in the art would have had to take a course of action in direct contravention to that taught by Lee *et al.*– she would have had to make the dye molecules *bigger*. Doing the *exact opposite* of what is taught and/or suggested by the prior art simply cannot be characterized as "obvious."

3.4 Conclusion

For the reasons stated above, Applicants submit the Lee *et al.* reference fails to render amended Claim 1 *prima facie* obvious. Since Claims 2-37 ultimately depend from amended Claim 1, the Lee *et al.* reference likewise fails to render these claims *prima facie* obvious. Accordingly, Applicants respectfully request that the rejection of Claims 1-37 under 35 USC § 103(a) be withdrawn.

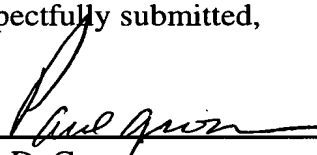
Conclusion

Applicants submit Claims 1-37, as amended, satisfy all of the criteria for patentability and are in condition for allowance. The cited Lee *et al.* reference does not teach or suggest the instantly claimed compounds, and in fact teaches away from making the substitutions necessary to arrive at the instantly claimed compounds. An early indication of allowance is therefore kindly requested.

No fee is believed due in connection with this Amendment. However, the Commissioner is authorized to charge any required fee or credit any overpayment to PE Biosystems Deposit Account No. 01-2213.

Respectfully submitted,

Date 4-7-99



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Enclosure

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DEOXYNUCLEOSIDE PHOSPHORAMIDITES—A NEW CLASS OF KEY INTERMEDIATES FOR DEOXYPOLYNUCLEOTIDE SYNTHESIS

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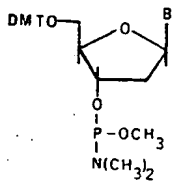
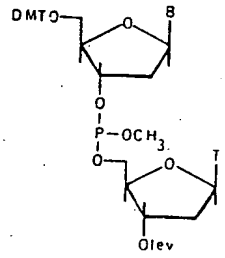
The development of a new class of nucleoside phosphites is described. These compounds are stable to normal laboratory conditions, are activated by mild acid treatment, and are observed to react essentially quantitatively with protected nucleosides.

A recent, key innovation in oligonucleotide synthesis was the introduction of the phosphite coupling approach by Letsinger and coworkers (1-3). This approach has been adapted to the synthesis of deoxyoligonucleotides (4-8), oligoribonucleotides (9-12), and nucleic acid analogs (13-15). Generally the approach involves the reaction of a suitably protected nucleoside, a bifunctional phosphitylating agent such as methoxydichlorophosphine, and a second protected nucleoside. Mild oxidation using iodine in tetrahydrofuran, lutidine and water generates the natural internucleotide bond. By varying the oxidation procedure, phosphorus analogs such as selenophosphates (14), imidophosphates (14) and thiophosphates (14, 15) can be generated. A serious limitation of this methodology, however, has been the instability of the reactive intermediates (nucleoside phosphomonochloridites or monotetrazolides) towards hydrolysis and air oxidation. This problem has been circumvented by either preparing the reactive species immediately prior to use or storing the active phosphite as a precipitate in hexanes at -20°C. We have recently solved this problem by synthesizing a new class of nucleoside phosphites that are easy to prepare by standard organochemical procedures, are stable under normal laboratory conditions to hydrolysis and air oxidation, and are stored as dry, stable powders. These key intermediates are N, N-dimethylaminophosphoramidites of the appropriately protected deoxynucleosides and are



Ia-d



IIIa-d

Ia, IIIa, B = 1-Thyminyl
Ib, IIIb, B = 1-(N-4-Benzoylcytosinyl)
Ic, IIIc, B = 9-(N-6-Benzoyladeninyl)
Id, IIId, B = 9-(N-2-Isobutyrylguaninyl)
lev = levulinyl
DMT = Di-*p*-anisylphenylmethyl

represented as compounds Ia-d. This communication outlines the synthesis, characterization, and reactivity of these phosphoramidites.

The synthesis of compounds Ia-d begins with the preparation of chloro-N, N-dimethylamino-methoxyphosphine [$CH_3O\ P(Cl)\ N(CH_3)_2$] which is used as a monofunctional phosphitylating agent. A 250 ml addition funnel was charged with 100 ml of precooled anhydrous ether (-78°C) and precooled (-78°C) anhydrous dimethylamine (45.9 g, 1.02 mol). The addition funnel was wrapped with aluminum foil containing dry ice in order to avoid evaporation of dimethylamine. This

solution was added dropwise at -15°C (ice-acetone bath) over 2 h to a mechanically stirred solution of methoxydichlorophosphine (16) (47.7 ml, 67.32 g., 0.51 mol) in 300 ml of anhydrous ether. The addition funnel was removed and the 1 ℓ, three-necked round bottom flask was stoppered with serum caps tightened with copper wire. The suspension was mechanically stirred for 2 h at room temperature. The suspension was filtered and the amine hydrochloride salt was washed with 500 ml anhydrous ether. The filtrate and washings were combined and ether was distilled at atmospheric pressure. The residue was distilled under reduced pressure. The product was collected at 40-42°C @ 13 mm Hg and was isolated in 71% yield (51.1 g, 0.36 mol). d^{25} = 1.115 g/ml. ^{31}P-N.M.R., δ = -179.5 ppm ($CDCl_3$) with respect to internal 5% v/v aqueous H_3PO_4 standard. ^{1}H-N.M.R. doublet at 3.8 and 3.6 ppm J_{P-H} = 14 Hz (3H, OCH_3) and two singlets at 2.8 and 2.6 ppm (6H, $N(CH_3)_2$). The mass spectrum showed a parent peak at m/e = 141.

The key intermediates Ia-d were prepared by the following procedure. 5'-O-Di-*p*-anisyl phenylmethyl nucleoside (1 mmol) was dissolved in 3 ml of dry, acid free chloroform and diisopropylethylamine (4 mmol) in a 10 ml reaction vessel preflushed with dry nitrogen. [$CH_3OP(Cl)N(CH_3)_2$] (2 mmol) was added dropwise (30-60 sec) by syringe to the solution under nitrogen at room temperature. After 15 min the solution was transferred with 35 ml of ethyl acetate into a 125 ml separatory funnel. The solution was extracted four times with an aqueous saturated solution of NaCl (80 ml). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to a foam under reduced pressure. The foam was dissolved with toluene (10 ml) (Id was dissolved with 10 ml of ethyl acetate) and the solution was added dropwise to 50 ml of cold hexanes (-78°C) with vigorous stirring. The cold suspension was filtered and the white powder was washed with 75 ml of cold hexanes (-78°C). The white powder was dried under reduced pressure and stored under nitrogen. Isolated yields of compounds Ia-d were 90-94% (see Table I).

TABLE I

COMPOUND	δ-^{31}P (ppm) (Acetone-d_6)	δ-^{31}P (ppm) ($CDCl_3$)	ISOLATED YIELD (%)
Ia	-146.0, -145.4	-147.7, -146.8	93, 95*
Ib	-146.3, -145.5	-148.0, -147.0	92, 95*
Ic	-146.1, -145.8	-147.4, -147.3	90, 98*
Id	-145.9, -145.7	-147.7, -147.2	90, 98*
IIIa	-139.6, -138.9	-140.8, -139.9	97**
IIIb	-139.6, -139.0	-140.6, -140.0	94**
IIIc	-139.7, -138.9	-141.0, -139.9	97**
IIId	-140.3, -140.2	-143.6, -141.9	93**

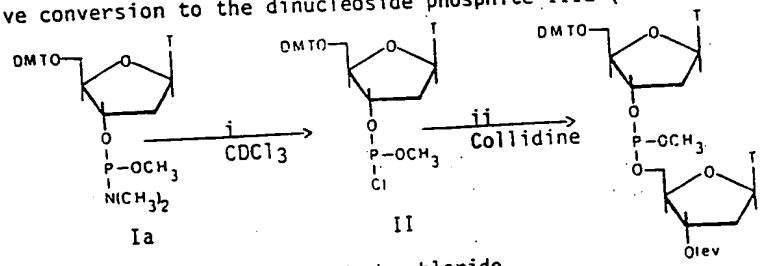
*Estimated purity from ^{31}P-N.M.R.

**Estimated yield from ^{31}P-N.M.R.

The purity of the products was checked by ^{31}P-N.M.R. Additionally, when analyzed by ^{31}P-N.M.R., these compounds were stable for at least a month when stored at room temperature under nitrogen. Furthermore, no significant amount of 3'-3' dinucleoside phosphite was detected by ^{31}P-N.M.R. (less than 4%). The low content of the 3'-3' dinucleoside phosphite was expected and represents a significant improvement over the original phosphite coupling procedure where a considerable amount of the unwanted 3'-3' dinucleoside phosphite was unavoidable (1-3, 9).

We have observed that mild acidic conditions can be used to activate Ia-d toward formation

of phosphite internucleotide bonds. These investigations were prompted by earlier research showing that aminophosphines can be protonated and therefore activated by acidic species (17-24). This activation process was initially monitored by ^{31}P-N.M.R. Thus, when N, N-dimethylaniline hydrochloride (1 mmol) in 0.5 ml of dry $CDCl_3$ was added at room temperature under nitrogen to Ia (0.5 mmol, -147.7 and -146.8 ppm) in 2 ml of dry, acid free $CDCl_3$ in a 10 mm N.M.R. tube, the chlorophosphite II (-167.2 ppm) was obtained in quantitative yield. Addition of 1.2 molar equivalents of 3'-O-levulinylthymidine (25) to the chlorophosphite II led to an essentially quantitative conversion to the dinucleoside phosphite IIIa (-140.8 and -139.9 ppm).



Ia II IIIa

i, N, N-Dimethylaniline hydrochloride
ii, 3'-O-Levulinylthymidine

Evidence supporting the assignment of the active chlorophosphite II to the peak at -167.2 was independently obtained by reacting 5'-O-Di-*p*-anisylphenylmethylthymidine with excess methoxydichlorophosphine (-181.6 ppm) in the presence of collidine in $CDCl_3$. The major reaction product as monitored by ^{31}P-N.M.R. was localized at -167.2 ppm.

Of the various weak acids investigated as potential activating agents, 1H-tetrazole fulfills all requirements. The compound is a non-hygroscopic, commercially available solid that can be easily purified and dried in one step by sublimation at 110°C @ 0.05 mm Hg. Activation by 1H-tetrazole was also monitored by ^{31}P-N.M.R. Thus, Ia (0.5 mmol) and 3'-O-levulinylthymidine (0.6 mmol) were placed in a 10 mm N.M.R. tube and sublimed 1H-tetrazole (1.5 mmol) in 2.5 ml of dry acetonitrile-d_3 was added under nitrogen atmosphere. The ^{31}P-N.M.R. spectrum was immediately recorded and displayed a quantitative yield of IIIa. Similar results were also obtained when Ib, Ic and Id were reacted with 3'-O-levulinylthymidine. The appropriate chemical shifts of compounds Ia-d and IIIa-d with respect to internal 5% v/v aqueous H_3PO_4 standard are reported in Table I. Complete physical and analytical properties of these compounds will be reported elsewhere.

The applicability of these reagents to the synthesis of deoxyoligonucleotides on polymer supports was also tested. Trial experiments were completed by condensing compounds Ia-d with N-2-isobutyryldeoxyguanosine attached covalently to silica gel. Thus, N-2-isobutyryldeoxyguanosine (1 μmole) covalently attached to silica gel (20 mg) at the 3'-position, Ia (10 μmole), and 1H-tetrazole (50 μmole in 0.1 ml dry acetonitrile) were shaken for 20 min and the reaction was then quenched with aqueous lutidine. The same reaction sequence was completed with Ib, Ic and Id. After the usual oxidation and deprotection procedures (8), d(TpG), d(CpG), d(ApG) and d(GpG) were obtained in 100%, 98%, 94%, and 93% yield respectively (measured spectrometrically from the dimethoxytrityl cation using an extinction of 7 X 10^4 at 498 nm). These dinucleotides were completely degraded by snake venom phosphodiesterase and the appropriate nucleosides and

nucleotides were obtained in the proper ratios (monitored via high pressure liquid chromatography analysis of snake venom phosphodiesterase hydrolysates).

The N, N-dimethylamino phosphines Ia-d therefore display tremendous potential in oligodeoxynucleotide synthesis. These compounds are easy to prepare and are stable to normal laboratory conditions. They are readily activated via protonation and condense with appropriate nucleosides to form internucleotide bonds in very high yields.

Acknowledgements

This is paper 5 in a series on Nucleotide Chemistry. This work was supported by grants from the National Institutes of Health (GM21120 and GM25680). S. Beaucage acknowledges a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada. M. Caruthers was supported by a Career Development Award from the National Institutes of Health (1 K04 GM00076).

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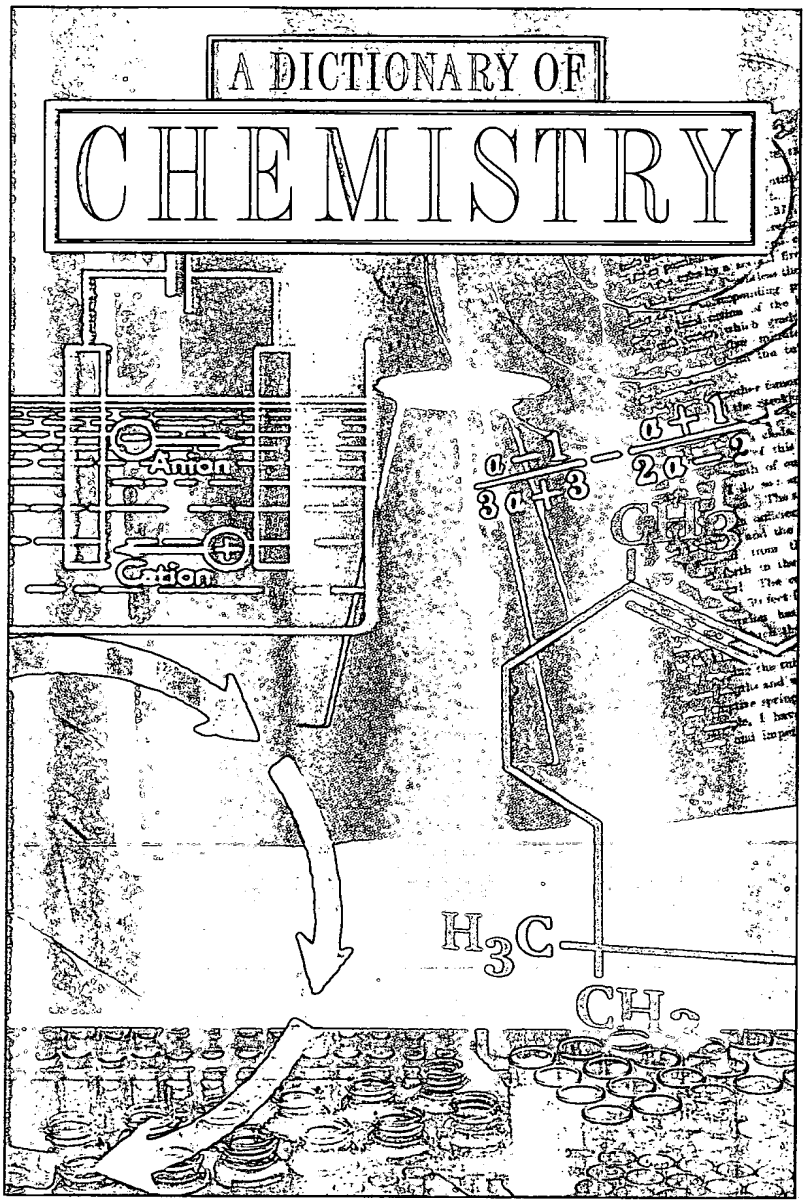

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Preface

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histochemistry The study of the distribution of the chemical constituents of tissues by means of their chemical reactions. It utilizes such techniques as staining, light and electron microscopy, autoradiography, and chromatography.

hole **1.** A vacant electron position in the lattice structure of a solid that behaves like a mobile positive *charge carrier. **2.** A vacant electron position in one of the inner orbitals of an atom.

holmium Symbol Ho. A soft silvery metallic element belonging to the *lanthanoids; a.n. 67; r.a.m. 164.93; r.d. 8.795 (20°C); m.p. 1474°C; b.p. 2695°C. It occurs in apatite, xenotime, and some other rare-earth minerals. There is one natural isotope, holmium–165; eighteen artificial isotopes have been produced. There are no uses for the element, which was discovered by P. T. Cleve and J. L. Soret in 1879.

HOMO *See* **highest occupied molecular orbital.**

homocyclic *See* **cyclic.**

homogeneous Relating to only one phase, e.g. a homogeneous mixture, a homogeneous *catalyst. *Compare* **hetereogeneous.**

homologous series A series of related chemical compounds that have the same functional group(s) but differ in formula by a fixed group of atoms. For instance, the simple carboxylic acids: methanoic (HCOOH), ethanoic (CH_3COOH), propanoic (C_2H_5COOH), etc., form a homologous series in which each member differs from the next by CH_2. Successive members of such a series are called *homologues*.

homolytic fission The breaking of a bond in a compound in which the fragments are uncharged free radicals. For example, $Cl_2 \rightarrow Cl\cdot + Cl\cdot$. *Compare* **heterolytic fission.**

homonuclear Denoting a molecule in which the atoms are of the same element.

homopolar bond *See* **chemical bond.**

homopolymer *See* **polymer.**

hormone A substance that is manufactured and secreted in very small quantities into the bloodstream by an endocrine gland or a specialized nerve cell and regulates the growth or functioning of a specific tissue or organ in a distant part of the body. For example, the hormone insulin controls the rate and manner in which glucose is used by the body.

hornblende Any of a group of common rock-forming minerals of the amphibole group with the generalized formula $(Ca,Na)_2(Mg,Fe,Al)_5(Al,Si)_8O_{22}(OH,F)_2$. Hornblendes consist mainly of calcium, iron, and magnesium silicate.

HPLC *See* **high-performance liquid chromatography.**

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